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Research Article

Effect of dietary L-glutamic acid on the growth performance, antioxidant status, flesh quality of rainbow trout (*Oncorhynchus mykiss*)

Mohammadzadeh B.[1*], Jafaryan H.[1], Adineh H.[1], Rahnama Kamamardkhi Y.[1]

1Department of Fisheries, Faculty of Agriculture and Natural Resources, Gonbad Kavous University, Gonbad Kavous, Iran
*Correspondence: Behrooz9@gmail.com; b.mohammadzadeh@gonbad.ac.ir

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Abstract

This study evaluated the effect of dietary L-glutamic acid supplementation in four dietary treatments, including Control (without added L-glutamic acid), 0.5, 1, and 2% L-glutamic acid added to the base diet, on rainbow trout (*Oncorhynchus mykiss*) juveniles (15-16 g). At the end of the 60-day culture period, growth performance, antioxidant status, amino acid composition, and muscle quality were assessed. The results showed that the 1% L-glutamic acid diet produced the highest weight gain (101.29 g) and the lowest feed conversion ratio (0.71) ($p\geq0.05$). The fish fed the diet supplemented with 0.5% exhibited the highest activities of glutathione peroxidase, superoxide dismutase, and catalase, as well as the highest glutathione levels ($p\leq0.05$). However, dietary treatments had no significant effect on the proximate composition of muscle tissue. Similarly, dietary L-glutamic acid did not markedly alter the amino acid composition of muscle. The highest ΔE (7.86, color difference compared with the control) and muscle hardness (11.68 N) were observed in fish fed a 2% L-glutamic acid diet ($p\geq0.05$), whereas the highest water-holding capacity (90.39%) and lowest cooking loss (23.32%) ($p\geq0.05$) were recorded in the 1% group. Overall, dietary supplementation with L-glutamic acid could improve muscle antioxidant status (at 0.5%) and growth performance and muscle quality (at 1 and 2%) of rainbow trout.

Introduction

Dietary factors play a crucial role in determining fish muscle quality by influencing tissue composition and nutritional characteristics. Nutrient intake can affect muscle texture, color, and nutritional value through changes in protein and lipid deposition and fatty acid profiles (Naylor *et al.,* 2000; Matos *et al.,* 2017). In addition, dietary supplements, including amino acids and vitamins, are commonly incorporated into formulated feeds to address nutritional deficiencies and may improve growth performance and flesh quality (Craig and Helfrich, 2002; Salamanca *et al.,* 2025). Amino acids are essential for protein synthesis and growth. Essential amino acids meet primary nutritional requirements, whereas non-essential amino acids contribute to intermediary metabolism (Li *et al.,* 2021; Xing *et al.,* 2024). Amino acids can also contribute to antioxidant defense through glutathione biosynthesis and the reduction of reactive oxygen species in stressed fish (Ciji and Akhtar, 2021; Salamanca *et al.,* 2025). As aquaculture intensifies, fish are exposed to multiple stressors that can impair growth, health, and product quality, increasing the importance of targeted nutritional interventions such as amino acid supplementation. Tryptophan, an essential amino acid and precursor of serotonin, can reduce oxidative stress markers in rohu (*Labeo rohita*) under thermal stress by increasing superoxide dismutase (SOD) and catalase (CAT) activities (Kumar *et al.,* 2014). Methionine and taurine have also been shown to suppress oxidative stress in seabream (*Acanthopagrus schlegelii*) by scavenging reactive oxygen species (ROS) (Yang *et al.,* 2024).

Beyond their metabolic functions, amino acids can contribute to improved fillet quality. Their inclusion in diets has been associated with improved sensory and nutritional attributes (Andersen *et al.,* 2016). Previous studies have shown that glutamate and glutamine can improve fish fillet firmness, an important quality attribute, by influencing muscle cell proliferation (Ingebrigtsen *et al.,* 2014; Østbye *et al.,* 2018). Dietary glutamate can also enhance fillet quality in Atlantic salmon (*Salmo salar*) (Larsson, 2014), indicating that both amino acid identity and species context may influence fillet-quality responses. Among amino acids, L-glutamate is of particular interest because of its potential role in modulating immune and oxidative responses through its involvement in reduced glutathione synthesis (Li *et al.,* 2020). Dietary glutamate has also been reported to influence muscle amino acid composition and sensory quality in fish flesh (Cai *et al.,* 2025). Glutamate serves as a precursor for glutathione, an important non-enzymatic antioxidant. Therefore, dietary glutamate supplementation may help buffer the oxidative burden associated with stress, providing a rationale for assessing antioxidant status in the present study. Glutamic acid, its ionized form glutamate, and the biochemically distinct amino acid glutamine are frequently discussed together in the literature, but they should be distinguished. L-glutamic acid is the free amino acid, whereas glutamate is its physiologically predominant ionized form; the two terms are often used

interchangeably in fish nutrition. Glutamine, in contrast, is synthesized from glutamate and ammonia by glutamine synthetase and is metabolically and functionally distinct. It has particularly important roles in intestinal energy metabolism and ammonia detoxification (Li *et al.,* 2020). Accordingly, findings from glutamine-supplementation studies are considered only for comparison where relevant and are not assumed to be directly interchangeable with the effects of glutamic acid evaluated in the present study.

Recent research has evaluated dietary glutamine in relation to growth and blood status in rainbow trout (Öz *et al.,* 2024). However, the specific effects of dietary L-glutamic acid on muscle texture, water retention characteristics, including water-holding capacity and cooking loss, antioxidant status, and muscle amino acid composition remain insufficiently characterized in *O. mykiss*. Feeding gibel carp (*Carassius gibelio*) with 2–3% glutamate has been reported to improve several fillet-quality parameters, including hardness, muscle springiness, thickness, density, myofiber number, collagen content, and sensory properties (Cai *et al.,* 2025). However, few studies have examined the combined effect of dietary L-glutamate on growth, antioxidant capacity, and flesh quality, particularly in rainbow trout (Li *et al.,* 2020; Li *et al.,* 2021). A better understanding of glutamate functions could support nutritional strategies in aquaculture and potentially reduce economic losses associated with poor product quality (Xing *et al.,* 2024). Previous research has reported that dietary inclusion of glutamic acid at levels up to 3% is safe and can improve growth, survival, and health-related parameters in fish (Li *et al.,* 2020). Based on this reported range, the present study selected dietary L-glutamic acid inclusion levels of 0.5%, 1%, and 2% (Li *et al.,* 2020; Öz *et al.,* 2024). Therefore, the present study aimed to investigate the effects of different dietary levels of L-glutamic acid on growth performance, antioxidant status, flesh quality, and muscle amino acid composition in rainbow trout. In addition, muscle antioxidant enzyme activities and instrumental quality attributes, including texture, color, water-holding capacity, and cooking loss, were evaluated to provide a comprehensive assessment of the potential effects of dietary L-glutamic acid on fish performance and muscle quality.

Materials and methods

All experimental procedures involving fish were conducted in accordance with institutional guidelines for animal care and use. Fish were euthanized by overdose anesthesia with MS-222, followed by spinal cord transection to minimize pain and distress.

Experimental Fish and Culture Conditions

Juvenile rainbow trout (15–16 g) were obtained from a commercial hatchery in Golestan Province, Iran. The fish were transported to the aquatic laboratory and acclimated to laboratory conditions for 10 days in a 400-L holding tank with 15±1°C, pH 8.31O_2 8.73 mg/L. Subsequently, the fish were randomly assigned to four experimental treatments, each with three replicates. Three rearing tanks (60-L

capacity) were used for each treatment, with each tank considered one experimental replicate. Fifteen juveniles were stocked in each tank. Water quality parameters, including pH, temperature, and dissolved oxygen, were measured daily throughout the experiment. Water was exchanged twice daily, and continuous aeration was maintained. During the 10-day acclimation period, the fish were fed a commercial trout feed (Faradaneh Company, Iran) with proximate composition shown in Table 1, three times daily at 3% of biomass. The fish were then fed the four experimental diets by hand for 60 days. Throughout the feeding trial, fish were hand-fed to apparent satiation three times daily at 09:00, 13:00, and 17:00, as described in the following subsection. The commercial diet was not used during the experimental feeding period. At the end of the feeding trial, growth performance was determined using tank-level production data. Five fish were randomly selected from each tank for morphometric measurements, including individual body weight, total length, liver weight, and visceral weight, which were used to calculate growth performance. Each tank was considered the experimental unit for growth and biochemical analyses. At the end of the culture period, five fish were randomly selected from each tank for analysis of muscle quality, including proximate composition, amino acid composition, antioxidant status, and other muscle-quality parameters. The five fish from each of the three replicate tanks within each treatment were collectively used to obtain a 15-fish muscle sample per treatment. Fish were deeply anesthetized with an overdose of tricaine Methane-Sulfonate (MS-222; >300 mg L^{-1}), buffered with sodium bicarbonate, and subsequently euthanized by spinal cord transection before tissue collection. Skinless dorsal muscle was then dissected and pooled by treatment before homogenization for proximate composition, amino acid, and antioxidant analyses.

Experimental diet supplementation and feeding trial

Four experimental diets were formulated and prepared in the laboratory according to standard aquafeed preparation procedures for juvenile salmonids (Belghit *et al.,* 2023). A commercial trout diet was used as the basal feed formulation. The basal feed ingredients (Table 1) were finely ground and thoroughly dry-mixed before supplementation with crystalline L-glutamic acid (Sigma, 6106-04-3, China) at 0 (Control), 5 (0.5%), 10 (1.0%), and 20 (2.0%) g/kg diet. L-Glutamic acid was added by replacing equivalent amounts of corn flour to maintain the overall diet formulation. The diets were pelleted using a laboratory extruder, air-dried at 50°C until a constant weight was achieved, and stored at –20 °C until use. Each dietary treatment was assigned to three replicate tanks, with 15 fish per tank. Water quality parameters were measured three times daily and maintained within optimal ranges for rainbow trout culture, including a temperature of 12–14°C, dissolved oxygen >7 mg/L, and pH 7.2–7.6. Fish were hand-fed the experimental diets three times daily at 09:00, 13:00, and 17:00 for 60 days.

Table 1: Ingredients (g/kg diet on dry matter basis) and chemical composition (g/100g^{-1} dry matter basis) of the experimental diets.

Ingredients (g/kg diet)	D1 (0%)	D2 (0.5%)	D3 (1%)	D4 (2%)
Fishmeal[1]	100	100	100	100
Poultry byproduct[2]	250	250	250	250
Soybean meal	210	210	210	210
Wheat meal	300	300	300	300
Sunflower oil	9	9	9	9
Soybean oil	7	7	7	7
Corn flour	109	104	99	89
L-Lysine	5	5	5	5
L-Methionine	5	5	5	5
Vitamin premix[a]	2.5	2.5	2.5	2.5
Mineral premix[b]	2.5	2.5	2.5	2.5
L-glutamic acid	0	5	10	20
Analyzed Proximate composition (g/100g-1 Wet matter basis)				
Protein	38.5	38.7	38.9	38.9
Lipids	7.78	7.72	7.70	7.69
Ash	7.45	7.38	7.41	7.45
Carbohydrate (Fiber)	37.17	37.05	36.99	37.02
Moisture	9.1	9.15	9.0	8.94

1- Pars kilka Co., Mazandaran, Iran (Kilka powder analysis; Protein: 70-72%, Fat: 8-11%, Ash: 11.6%, Moisture: 7-9%). 2- Morghenojan.Co., Tehran, Iran (Crude protein 63 %; crude fat 16 %; crude ash 6 %; crude fiber 4 %.). [a] Vitamin premix (per kg of diet): vitamin A, 2000 IU; vitamin B_1 (thiamin), 5 mg; vitamin B_2 (riboflavin), 5 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.025 mg; vitamin D_3, 1200 IU; vitamin E, 63 mg; vitamin K_3, 2.5 mg; folic acid, 1.3 mg; biotin, 0.05 mg; pantothenic acid calcium, 20 mg; inositol, 60 mg; ascorbic acid (35%), 110 mg; niacinamide, 25 mg. [b] Mineral premix (per kg of diet): $MnSO_4$, 10 mg; $MgSO_4$, 10 mg; KCl, 95 mg; NaCl, 165 mg; $ZnSO_4$, 20 mg; KI, 1 mg; $CuSO_4$, 12.5 mg; $FeSO_4$, 105 mg; Co, 1.5 mg.

Growth performance

At the end of the experiment, growth performance was determined using the following equations:

$$Weight\ Gain\ (WG) = Final\ body\ weight\ (FBW) - initial\ body\ weight\ (IBW)$$

$$\text{Weight Gain (\%WG)} = \frac{Final\ body\ weight\ (g) - Initial\ body\ weight\ (g)}{Initial\ body\ weight\ (g)} \times 100$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{Feed\ intake\ (FI)}{Weight\ gain\ (WG)}$$

$$\text{Specific Growth Rate (SGR)(\%day)} = 100 \times \frac{\ln Final\ body\ weight\ (FBW) - \ln Intial\ body\ weight\ (IBW)}{t}$$

$$Condition\ Factor\ (CF): \frac{Final\ weight\ (g)}{Length\ fish(cm)^3} \times 100$$

$$\text{Hepatosomatic Index (HSI\%)} = \frac{Liver\ weight\ (g)}{Total\ body\ weight\ (g)} \times 100$$

$$\text{Viscerosomatic Index (VSI\%)} = \frac{Viscera\ weight\ (g)}{Total\ body\ weight\ (g)} \times 100$$

Muscle Antioxidant Status

To determine muscle antioxidant status, dorsal muscle samples were collected on day 60 immediately after fish were removed from the rearing tanks and stored at -80°C until analysis. Muscle tissue was homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 12,000

× g for 20 min at 4°C. The supernatant was used to determine antioxidant enzyme activities. Muscle malondialdehyde (MDA) and glutathione (GSH) levels were measured according to the methods described by Zhang *et al*. (2008) and Deng *et al*. (2012), respectively. Muscle antioxidant enzymes, including catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD), were analyzed according to the methods described by Jiang *et al.* (2010), Tan *et al.* (2010), and Jiang *et al*. (2013), respectively.

Proximate composition

The proximate composition of skinless dorsal muscle, including moisture, crude protein, crude lipid, and ash, was determined according to standard AOAC procedures. Moisture and ash contents were determined according to AOAC (2000) methods 925.45 and 923.03, respectively. Moisture content was determined by drying samples at 105°C for 24 h, whereas ash content was determined by incinerating samples at 550°C for 24 h in an electric furnace. Crude protein and crude lipid contents were determined using the Kjeldahl and Soxhlet methods, respectively, according to AOAC (2000) methods 981.10 and 996.06. Total carbohydrate content (%) was calculated by difference as follows: 100 − (protein + lipid + ash + moisture). Gross energy (kJ per 100 g) was calculated using conversion factors of 23.6 kJ/g for protein, 39.5 kJ/g for lipid, and 17.2 kJ/g for carbohydrate (NRC, 1993; Moniruzzaman, 2021).

Amino acid analysis

Amino acid concentrations in fish muscle were determined according to the method described by Mohammadzadeh and Feizy (2025). Briefly, 0.2 g of fish muscle was hydrolyzed with 6 M/L hydrochloric acid for 20 h at 110 °C under a nitrogen atmosphere. After hydrolysis, the sample was diluted with borate solution (125 mM, pH 9.4). Then, 200 µL of borate solution, 800 µL of methanol (98%), and 50 µL of L-homoserine solution (1 mM/L) were added to a test tube and vortexed for 20 s. Subsequently, 250 µL of the resulting solution, 100 µL of borate solution (125 mmol/L, pH 9.4), and 50 µL of OPA solution were added to another tube and vortexed for 2 min. Next, 25 µL of 0.75 M hydrochloric acid was added to the mixture. Finally, 50 µL of the resulting solution was mixed with 200 µL of borate solution (125 mM/L, pH 9.4) for several seconds and injected into a high-performance liquid chromatography (HPLC) system equipped with a fluorescent LC350 detector (Young Lin, South Korea). The HPLC system was fitted with a C18 column (15 cm × 4.6 mm, 5 µm particle size) and operated at 35°C with a mobile-phase flow rate of 1.3 mL/min. Total amino acid concentrations were expressed as mg/g dry weight of sample.

Muscle quality parameters

To evaluate the effects of dietary L-glutamate supplementation on muscle quality, muscle pH, color, texture, water-holding capacity (WHC), and cooking loss were measured at the end of the rearing period after skinless muscle samples were

collected. Three fish from each treatment group were analyzed using a colorimeter (CHROMA METER CR-40). Muscle color was characterized based on *L**, *a**, and *b** values, where *L** represents lightness, *a** represents the red–green axis, and *b** represents the yellow–blue axis. Hue, chroma, overall color difference (ΔE), and the redness index were calculated using the following equations (Choubert and Baccaunaud, 2006; Herath *et al.,* 2016):

$$\text{Hue} = \text{Arctan}(*b/a*),\ \text{Chroma} = (*a^2 + b^2)^{0.5},\ \text{Redness} = (*b/a*),\ \Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2)]^{0.5}$$

Muscle texture was evaluated using a texture analyzer (CT3 Brookfield, American) on samples measuring 3 cm × 2 cm × 1 cm. The samples were compressed using a 4-cm-diameter probe at a force of 0.5 Newtons and a speed of 1 mm per second. Hardness, springiness, chewiness, and cohesiveness were determined from the force–deformation curves, with each measurement performed in triplicate. Water-holding capacity (WHC) was determined by centrifuging minced muscle at 5,000 × g for 10 min, as described by Xu *et al.* (2019). Cooking loss was calculated as the difference between the sample weight before and after heating at 90°C for 10 min (Brinker and Reiter, 2011). Muscle pH was measured using a digital pH meter.

Statistical analysis

For growth performance and biochemical parameters, each tank (n=3 tanks per diet) was considered the experimental unit. Data from individual fish were averaged within each tank before statistical analysis to avoid pseudo-replication. Statistical analyses were performed using SPSS version 21.0. Data normality was assessed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was used to evaluate differences among dietary treatments in growth performance, antioxidant status, chemical composition, amino acid composition, and muscle quality parameters. When significant differences were detected, means were compared using Duncan's post hoc test. Each assay was performed using three replicates. Data are presented as mean±S.D., and statistical significance was set at $p<0.05$.

Results

Growth performance

The growth performance and feed utilization indices of rainbow trout fed different levels of L-glutamic acid for 60 days are presented in Table 2. Initial body weight did not differ significantly among treatments ($p\geq0.05$). At the end of the rearing period, final body weight, weight gain, percentage weight gain, specific growth rate, and protein efficiency ratio were significantly higher ($p\leq0.05$) in fish fed the 1% and 2% L-glutamic acid diets than in those fed the control and 0.5% L-glutamic acid diets. Conversely, the feed conversion ratio was significantly lower ($p\leq0.05$) in fish fed the 1% L-glutamic acid diet than in the other groups. The

highest condition factor (1.04±0.02) was recorded in the control group.

Muscle antioxidant status

The muscle antioxidant capacity and status of rainbow trout fed different levels of L-glutamic acid are presented in Table 3. Glutathione peroxidase activity was significantly higher ($p \leq 0.05$) in the control and 0.5% L-glutamic acid groups than in the 1% and 2% L-glutamic acid. Superoxide dismutase activity was significantly higher ($p \leq 0.05$) in the 0.5% L-glutamic acid group (2.19±0.08 U/mg protein) than in the other groups. Malondialdehyde (MDA) content was significantly higher ($p \leq 0.05$) in the 2% L-glutamic acid group (6.34±0.1 nmol/mg protein) than in the other groups. Catalase activity (2.00±0.02 U/mg protein) and glutathione (GSH) content (211.05±1.75 nmol/mg protein) were significantly higher ($p \leq 0.05$) in the 0.5% L-glutamic acid group than in the other treatment groups.

Table 2: Growth performance and feed utilization of rainbow trout fed the experimental diet.

Growth Parameters	Feed diet experimental			
	Control	Glutamic acid 0.5%	Glutamic acid 1%	Glutamic acid 2%
Initial Weight (g)	16.13 ± 0.61	16.12 ± 1.01	15.04 ± 0.14	15.56 ± 0.15
Final Weight (g)	93.08 ± 1.08^{b}	96.63 ± 3.35^{b}	116.33 ± 0.33^{a}	115.50 ± 14.50^{a}
Weight Gain (g)	76.95 ± 0.47^{b}	80.51 ± 4.36^{b}	101.29 ± 0.19^{a}	99.94 ± 14.35^{a}
Percent Weight Gain (%)	477.44 ± 15.15^{b}	501.80 ± 58.78^{b}	673.50 ± 5.00^{a}	641.74 ± 86.04^{a}
Specific Growth Rate (%)	2.92 ± 0.04^{b}	2.98 ± 0.16^{b}	3.41 ± 0.01^{a}	3.33 ± 0.19^{a}
Condition Factor	1.04 ± 0.02^{a}	0.97 ± 0.03^{b}	1.02 ± 0.00^{ab}	0.98 ± 0.02^{b}
Feed Conversion Ratio	0.81 ± 0.01^{ab}	0.83 ± 0.04^{a}	0.71 ± 0.00^{b}	0.76 ± 0.11^{ab}
Protein Efficiency Ratio	3.01 ± 0.02^{b}	3.15 ± 0.17^{b}	3.97 ± 0.06^{a}	3.91 ± 0.56^{a}

Values presented as mean±S.D. (n=3). Means with different superscript letters are significantly different ($p \leq 0.05$). Among the different treatments.

Table 3: Indices of antioxidant status: protein (mg/g tissue), glutathione peroxidase (GPx), superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT), and Glutathione in the muscle of rainbow trout fed diets with varying levels of glutamic acid.

	Dietary experimental			
	Control	Glutamic acid 0.5%	Glutamic acid 1%	Glutamic acid 2%
GPx (U/mg protein)	13.87 ± 0.3^{a}	14.05 ± 0.35^{a}	13.15 ± 0.05^{b}	12.95 ± 0.05^{b}
SOD (U/mg protein)	1.78 ± 0.06^{b}	2.19 ± 0.08^{a}	1.80 ± 0.02^{b}	1.82 ± 0.02^{b}
MDA (nmol/mg protein)	5.68 ± 0.05^{c}	5.87 ± 0.09^{b}	5.67 ± 0.08^{c}	6.34 ± 0.1^{a}
CAT (U/mg protein)	1.90 ± 0.07^{b}	2.00 ± 0.02^{a}	1.86 ± 0.02^{b}	1.69 ± 0.01^{c}
Glutathione (nmol/mg protein)	193.1 ± 1.30^{b}	211.05 ± 1.75^{a}	196.75 ± 2.45^{b}	193.2 ± 2.70^{b}

Data are reported as mean ± standard deviation of three replicates. Different lowercase letters within a column indicate a significant difference ($p \leq 0.05$) among treatments.

Muscle chemical composition

The chemical composition and gross energy content of muscle from rainbow trout fed diets containing different levels of L-glutamic acid are presented in Table 4. No significant differences were observed among dietary treatments in any of the measured chemical components ($p \geq 0.05$). Numerically, the highest protein content (15.1±74.07% of total) was

observed in the 1% L-glutamic acid group. The highest fat (4.06±0.74%), moisture (76.16±0.47%), and ash (5.40±0.58%) contents were recorded in the 2% L-glutamic acid group. The highest carbohydrate content (3.39±0.72%) was observed in the control group.

Muscle amino acid analysis

The concentrations of individual amino acids in the muscle of rainbow trout fed diets containing different levels of L-glutamic acid are presented in Table 5.

Table 4: Chemical composition (protein, fat, moisture, ash, carbohydrate) and total energy content in the muscle of rainbow trout fed diets containing different levels of glutamic acid.

Proximate Composition	Feed diet experimental			
	Control	Glutamic acid 0.5%	Glutamic acid 1%	Glutamic acid 2%
Protein (%)	14.59 ± 1.16^{a}	15.09 ± 0.97^{a}	15.74 ± 1.07^{a}	15.12 ± 1.43^{a}
Lipid (%)	1.97 ± 1.04^{a}	2.69 ± 1.72^{a}	4.05 ± 1.62^{a}	4.06 ± 0.74^{a}
Moisture (%)	74.82 ± 1.26^{a}	75.78 ± 1.30^{a}	73.14 ± 2.77^{a}	76.16 ± 0.47^{a}
Ash (%)	5.23 ± 0.30^{a}	5.38 ± 0.78^{a}	5.01 ± 0.08^{a}	5.40 ± 0.58^{a}
Carbohydrate (%)	3.39 ± 0.72^{a}	1.73 ± 2.09^{a}	2.61 ± 4.07^{a}	0.31 ± 0.54^{a}
Total Energy	480.60 ± 17.98^{c}	492.06 ± 6.30^{bc}	576.54 ± 12.98^{a}	522.54 ± 12.62^{b}

Mean ± standard deviation. Proximate chemical composition values for protein, fat, moisture, and ash are expressed as a percentage of the total and base wet weight of the sample. Total energy is expressed in kJ per 100 g of muscle tissue. Data are presented as mean ± standard deviation of three replicates. Different lowercase letters in each row indicate a significant statistical difference between the different groups ($p \leq 0.05$).

The concentrations of histidine, isoleucine, leucine, phenylalanine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, serine, and tyrosine did not differ significantly among treatments ($p \geq 0.05$). Cysteine concentration was significantly lower ($p \leq 0.05$) in the control group (1.68±0.04 mg/100 g dry sample) than in other treatment groups. Glycine concentration was significantly lower ($p \leq 0.05$) in the 1% L-glutamic acid group (23.15±1.75 mg/100 g dry sample) than in other groups. In addition, lysine, methionine, and proline concentrations were significantly lower ($p \leq 0.05$) in the 1% L-glutamic acid group than in other treatment groups. The concentrations of total amino acids (TAA), total essential amino acids (TEAA), total non-essential amino acids (TNEAA), total branched-chain amino acids (BCAA), total aromatic amino acids (TAAA), total flavor-related amino acids, and total acidic amino acids did not differ significantly among treatments ($p \geq 0.05$). Total sulfur-containing amino acids were significantly lower ($p \leq 0.05$) in the 1% L-glutamic acid group (9.3±83.36 mg/100 g dry sample) than in other treatment groups. The highest total basic amino acid content was recorded in the 0.5% L-glutamic acid group (66.3±74.67 mg/100 g dry sample). The lowest ratios of essential to total amino acids (32.62±0.81%), essential to non-essential amino acids (48.43±1.79%), and leucine to isoleucine (3.17±0.48%) were observed in the 1% L-glutamic acid group. The lowest ratio of non-essential to total amino acids was observed in the 0.5% L-glutamic acid group (64.0±22.44%). In addition, the ratio of flavor-related amino acids to total amino

acids was significantly lower (p<0.05) in the 0.5% L-glutamic acid group than in the other treatment groups.

Muscle quality

The physical quality parameters, including color indices, texture profile analysis, water-holding capacity, and cooking loss, of rainbow trout muscle fed diets containing different levels of L-glutamic acid are presented in Table 6. The highest lightness value (L*) was observed in the 0.5% L-glutamic acid group. Yellowness (b*), redness (a*), hue, and chroma did not differ significantly among treatments (p≥0.05). The greatest total color difference relative to the control was recorded in the 2% L-glutamic acid group (7.86±6.49).

Table 5: Amino acid ($mg\ g^{-1}$ dry sample) analysis in the muscle of rainbow trout fed diets containing different levels of glutamic acid.

Amino Acid Composition	Feed diet experimental			
	Control	Glutamic acid 0.5%	Glutamic acid 1%	Glutamic acid 2%
Cysteine	1.68 ± 0.04^{b}	2.05 ± 0.09^{ab}	2.44 ± 0.73^{a}	1.98 ± 0.19^{ab}
Histidine	8.07 ± 3.30^{a}	9.62 ± 0.18^{a}	7.52 ± 2.24^{a}	10.17 ± 0.45^{a}
Isoleucine	7.35 ± 0.23^{a}	7.94 ± 0.66^{a}	10.41 ± 3.40^{a}	7.97 ± 1.02^{a}
Leucine	29.88 ± 2.60^{a}	27.34 ± 0.29^{a}	31.90 ± 6.92^{a}	28.50 ± 3.64^{a}
Lysine	32.68 ± 0.32^{a}	33.73 ± 1.36^{a}	23.18 ± 2.88^{b}	32.53 ± 2.74^{a}
Methionine	13.01 ± 0.28^{a}	13.70 ± 2.28^{a}	7.39 ± 3.90^{b}	12.89 ± 2.00^{a}
Phenylalanine	14.19 ± 1.35^{a}	14.58 ± 1.11^{a}	15.46 ± 2.82^{a}	14.30 ± 0.83^{a}
Threonine	11.25 ± 0.01^{a}	11.00 ± 1.36^{a}	10.55 ± 3.38^{a}	11.34 ± 1.20^{a}
Valin	13.27 ± 0.34^{a}	12.79 ± 0.47^{a}	19.09 ± 7.63^{a}	12.94 ± 1.14^{a}
Alanine	36.26 ± 0.80^{a}	35.03 ± 4.08^{a}	39.62 ± 8.74^{a}	34.37 ± 1.23^{a}
Arginine	24.31 ± 0.85^{a}	23.40 ± 2.50^{a}	26.68 ± 6.65^{a}	22.85 ± 1.76^{a}
Aspartic acid	43.76 ± 1.78^{a}	37.60 ± 5.79^{a}	48.38 ± 7.94^{a}	46.97 ± 5.31^{a}
Glutamic acid	70.73 ± 1.59^{a}	67.33 ± 0.04^{a}	70.96 ± 8.58^{a}	70.56 ± 6.04^{a}
Glycine	26.15 ± 0.65^{a}	23.88 ± 1.08^{b}	23.15 ± 1.75^{ab}	24.82 ± 1.34^{ab}
Proline	19.97 ± 0.47^{a}	18.42 ± 2.45^{a}	15.30 ± 1.09^{b}	18.26 ± 0.66^{a}
Serine	19.70 ± 0.55^{a}	17.59 ± 0.86^{a}	24.32 ± 6.62^{a}	18.91 ± 1.00^{a}
Tyrosine	16.31 ± 0.49^{a}	14.96 ± 1.36^{a}	16.83 ± 4.61^{a}	14.98 ± 0.20^{a}
ΣTAA	388.53 ± 5.27^{a}	370.92 ± 1.65^{a}	393.18 ± 62.10^{a}	384.33 ± 30.80^{a}
ΣEAA	131.36 ± 1.91^{a}	132.73 ± 1.05^{a}	127.93 ± 17.17^{a}	132.62 ± 13.22^{a}
ΣNAA	257.17 ± 7.18^{a}	238.19 ± 2.70^{a}	265.25 ± 44.95^{a}	251.71 ± 17.57^{a}
ΣBcAA	50.49 ± 3.19^{a}	48.07 ± 0.10^{a}	61.40 ± 17.92^{a}	49.42 ± 5.80^{a}
ΣArAA	30.50 ± 0.86^{a}	29.53 ± 0.25^{a}	32.28 ± 7.41^{a}	29.27 ± 1.03^{a}
ΣDAA	176.90 ± 4.82^{a}	163.83 ± 2.75^{a}	182.12 ± 26.53^{a}	176.72 ± 13.93^{a}
ΣBAA	65.05 ± 3.83^{ab}	66.74 ± 3.67^{a}	57.38 ± 4.81^{b}	65.55 ± 4.95^{ab}
ΣAAA	114.48 ± 3.36^{a}	104.92 ± 5.75^{a}	119.34 ± 16.49^{a}	117.53 ± 11.36^{a}
ΣSAA	14.69 ± 0.33^{a}	15.75 ± 2.38^{a}	9.83 ± 3.36^{b}	14.86 ± 2.19^{a}
EAA/TAA	33.81 ± 0.95^{bc}	35.78 ± 0.44^{a}	32.62 ± 0.81^{c}	34.47 ± 0.67^{ab}
NEAA/TAA	66.19 ± 0.95^{ab}	64.22 ± 0.44^{b}	67.38 ± 0.81^{a}	65.53 ± 0.67^{bc}
EAA/NEAA	51.12 ± 2.17^{bc}	55.73 ± 1.07^{a}	48.43 ± 1.79^{c}	52.61 ± 1.58^{ab}
DAA/TAA	45.52 ± 0.62^{a}	44.17 ± 0.94^{b}	46.39 ± 0.83^{a}	45.98 ± 0.06^{a}
Leu/Iso	4.06 ± 0.22^{a}	3.46 ± 0.32^{ab}	3.17 ± 0.48^{b}	3.57 ± 0.01^{ab}

Data are expressed as mean ± standard deviation of three replicates. ΣTAA: Total Amino Acids (mg/g of dry sample), ΣEAA: Total Essential Amino Acids (mg/g of dry sample), ΣNAA: Total Non-Essential Amino Acids (mg/g of dry sample), ΣBcAA: Total Branched-Chain Amino Acids (mg/g of dry sample), ΣArAA: Total Aromatic Amino Acids (mg/g of dry sample), ΣDAA: Total Delicious Amino Acids (mg/g of dry sample), ΣBAA: Total Basic Amino Acids (mg/g of dry sample), ΣAAA: Total Acidic Amino Acids (mg/g of dry sample), ΣSAA: Total Sulfur-Containing Amino Acids (mg/g of dry sample). EAA/TAA: Essential Amino Acids to Total Amino Acids as%, NEAA/TAA: Non-Essential Amino Acids to Total Amino Acids as%, EAA/NEAA: Essential to Non-Essential Amino Acids as%, DAA/TAA: Delicious Amino Acids to Total Amino Acids as%, Leu/Iso: Leucine to Isoleucine as %. Data on amino acid ratios are expressed as percentages of the total.

Texture profile analysis showed that the highest hardness (11.68±1.45 N), gumminess (2.09±0.49 N), and chewiness (1.40±0.67 mJ) were observed in the 2% L-glutamic acid group. The lowest adhesiveness (0.89±0.27 mJ) was recorded in the 0.5% L-glutamic acid group. The highest flexibility (0.12±0.05) was observed in the control group, whereas the greatest springiness (0.70±0.14 mm) was recorded in the 1% L-glutamic acid group. Cohesiveness did not differ significantly among treatments ($p \geq 0.05$). The highest water-holding capacity (90.39±2.30%) and lowest cooking loss (23.32±4.00%) were observed in samples from the 1% L-glutamic acid group.

Table 6: Physical quality parameters of muscle: color indices (L*, a*, and b*, hue, chroma, and total color change), texture profile analysis including hardness (N), adhesiveness (mJ), resilience (mm), cohesiveness (Ratio), springiness (mm), gumminess (N), and chewiness (mJ), water-holding capacity (%), and cooking loss (%) in the muscle of rainbow trout fed with different levels of L-glutamic acid.

Muscle Quality Indices	Feed diet experimental			
	Control	Glutamic acid 0.5%	Glutamic acid 1%	Glutamic acid 2%
*L**	21.54 ± 0.60^{ab}	21.83 ± 0.49^{a}	21.51 ± 0.77^{bc}	21.47 ± 0.85^{c}
*a**	8.65 ± 0.91^{a}	9.02 ± 0.35^{a}	7.89 ± 0.79^{a}	7.54 ± 0.83^{a}
*b**	31.15 ± 10.66^{a}	33.94 ± 1.86^{a}	33.82 ± 1.24^{a}	34.41 ± 1.73^{a}
Hue	1.24 ± 0.22^{a}	1.31 ± 0.02^{a}	1.34 ± 0.03^{a}	1.35 ± 0.03^{a}
Chroma	32.58 ± 9.78^{a}	35.12 ± 1.75^{a}	34.74 ± 1.06^{a}	35.24 ± 1.53^{a}
ΔE	0	5.65 ± 3.14^{a}	4.99 ± 3.88^{a}	7.86 ± 6.49^{a}
Hardness	10.36 ± 2.03^{ab}	8.77 ± 2.52^{b}	10.76 ± 1.71^{ab}	11.68 ± 1.45^{a}
Adhesiveness	1.35 ± 0.29^{a}	0.89 ± 0.27^{b}	1.08 ± 0.31^{ab}	1.03 ± 0.21^{b}
Springiness	0.12 ± 0.05^{a}	0.08 ± 0.02^{b}	0.08 ± 0.03^{b}	0.09 ± 0.03^{ab}
Cohesiveness	0.14 ± 0.04^{a}	0.12 ± 0.03^{a}	0.14 ± 0.03^{a}	0.15 ± 0.03^{a}
Resilience	0.58 ± 0.13^{ab}	0.48 ± 0.12^{b}	0.70 ± 0.14^{a}	0.65 ± 0.16^{a}
Gumminess	1.79 ± 0.65^{ab}	1.27 ± 0.61^{b}	1.79 ± 0.58^{ab}	2.09 ± 0.49^{a}
Chewiness	1.09 ± 0.52^{ab}	0.67 ± 0.48^{b}	1.23 ± 0.68^{ab}	1.40 ± 0.67^{a}
Water-Holding Capacity	86.96 ± 0.32^{b}	89.32 ± 0.82^{ab}	90.39 ± 2.30^{a}	89.74 ± 0.47^{a}
Cooking Loss	30.73 ± 11.28^{a}	30.54 ± 2.39^{a}	23.32 ± 4.00^{a}	27.04 ± 1.40^{a}

Data are presented as means of three replicates ± standard deviation. Different lowercase letters indicate significant differences ($p \leq 0.05$) among treatments (fish fed diets containing different levels of L-glutamic acid).

Discussion

Growth performance and feed conversion ratio (FCR) were improved at the 1% and 2% L-glutamic acid inclusion levels in the present study, consistent with previous reports. Zhelyazkov and Stratev (2019) reported enhanced growth performance in rainbow trout fed monosodium glutamate (MSG) at levels ranging from 0.5% to 5%, attributing this response mainly to gustatory palatability enhancement. The low FCR values observed in the present study in 1% and 2% L-glutamic acid diets may be attributed to the high digestibility of the experimental diets, favorable water quality, relatively small fish size, and the efficient nutrient utilization associated with L-glutamic acid supplementation. Similarly, Öz *et al.,* (2024) reported that dietary glutamine supplementation at 1.5% and 2% reduced FCR of rainbow trout from 0.83 to 0.69 and 0.65, respectively. This growth enhancement is primarily attributed to the role of glutamate as an

appetite stimulant and feed attractant, a mechanism reported to increase feed intake in other species. Glutamate also serves as a primary energy source for intestinal cells, thereby contributing to gut health and structural integrity (Jiang *et al.,* 2024). Yoshida *et al*. (2016) demonstrated that glutamate improves proximal intestinal structure in rainbow trout, particularly in diets containing plant-based protein, thereby enhancing nutrient absorption and, consequently, growth. Nevertheless, some studies have reported different outcomes. Belghit *et al*. (2023) in rainbow trout and Cai *et al*. (2025) in bighead carp observed no significant effect of glutamate supplementation on growth indices. These authors attribute this discrepancy to differences in feeding method (restricted vs. feeding to satiety), experimental duration, and basal diet composition. When the control diet already provides optimal nutritional value and palatability, additives such as glutamate may exert less pronounced effects (Cai *et al.,* 2025). It is worth noting that the high standard deviations (SD) observed for some of the growth performance indexes in the 2% L-glutamic acid group may reflect variation in growth responses among experimental replicates. Such variation can occur in fish due to differences in feeding behavior, competition, physiological condition, and nutrient utilization (Batzina *et al.,* 2020; Goodrich and Clark, 2023).

The key findings regarding the muscle antioxidant status of rainbow trout in this study indicate that a lower dietary L-glutamic acid level (0.5%) enhanced the activities of effective antioxidant enzymes as well as glutathione content, whereas a higher level (2%) increased malondialdehyde content. These results align with several studies on various fish species, although some discrepancies were noted. For example, Öz and İnanan (2025) reported that glutamine supplementation at 1.5% and 2% reduced malondialdehyde levels and improved oxidative status in female trout, consistent with the present findings at lower supplementation levels; this response likely reflects glutamine's role as a precursor of glutathione, which directly scavenges free radicals and prevents oxidant-induced apoptosis. In the present study, however, higher glutamic acid levels increased malondialdehyde content; this discrepancy may be attributable to species differences (trout vs. female trout) or to the focus on muscle tissue, in which high levels may disrupt oxidative balance. Zhao *et al*. (2019) demonstrated that glutamate increased glutathione content and the activities of Cu/Zn-SOD, glutathione peroxidase, glutathione-S-transferase, and glutathione reductase in Jian carp, enzymes regulated by the Nrf2 pathway. This finding is consistent with the increased glutathione content and enzymatic activities observed at moderate levels in the present study, possibly reflecting glutamate's role in enhancing protein synthesis and reducing protein degradation through associated metabolic pathways. Furthermore, Wang *et al*. (2025) reported that glutamate improved antioxidant capacity and increased glutamine synthetase activity in Nile tilapia under alkaline stress, consistent with the increased glutathione levels observed at lower concentrations in

the present study; higher levels, however, may increase oxidative stress through metabolic competition. In the current study, the improved antioxidant status observed in fish fed 0.5% L-glutamic acid may indicate that this supplementation level provided a more favorable balance for antioxidant defense. Glutamate contributes to the synthesis of glutathione (GSH), an important antioxidant involved in protecting cells against oxidative damage. On the other hand, Glutamate is a substrate for GSH, but only up to a point. Glutamate is one of three amino acids in GSH, and its availability can limit GSH synthesis in tissues (Zhao *et al.,* 2020). A modest supply therefore raises GSH. GSH synthesis is also limited by cysteine supply and by feedback inhibition from GSH itself, so more glutamate beyond a point cannot raise GSH further. This fits the plateau at 1–2%. In the present study, the 0.5% group showed the highest SOD, CAT, and GSH values, while the 2% group exhibited the highest malondialdehyde (MDA) concentration and the lowest CAT activity. These findings suggest that moderate supplementation may support antioxidant protection, whereas the higher inclusion level may be associated with increased lipid peroxidation. In addition, the MDA differences were small, and MDA at 0.5% was marginally above the control, so we interpret the 0.5% effect as enhanced antioxidant capacity (GSH, SOD, CAT) rather than reduced lipid peroxidation. Previous studies in fish have also reported dose-dependent responses to dietary glutamate (Jiang *et al.,* 2015; Zhao *et al.,* 2019, 2020). Nevertheless, because oxidative stress pathways and related gene expression were not directly measured, further investigation is needed to establish the mechanisms underlying these responses.

Consistent with whole-body findings reported for rainbow trout by Belghit *et al*. (2023), dietary L-glutamic acid supplementation did not alter muscle protein, lipid, moisture, or ash content, indicating that functional supplementation modulates cellular quality traits without altering macroscopic tissue composition. This lack of significant change may reflect the restricted feeding strategy used in that study and the nutrient adequacy of the basal diet in the present study; under such conditions, excess amino acids are likely catabolized for energy rather than stored as protein or fat. These results, however, contrast with several other studies that have reported significant effects. Zhao *et al*. (2019) in Jian carp and Ma *et al.* (2020) in grass carp observed significant increases in muscle protein and fat content following glutamate or glutamine supplementation. Similarly, Öz *et al.* (2025) reported that glutamine supplementation increased muscle protein content while decreasing fat content in female rainbow trout. These divergent outcomes suggest that the effect of L-glutamic acid on carcass composition depends on fish species, developmental stage, and dietary formulation. Changes in fat content, in turn, have been attributed to the regulation of lipid-metabolism-related gene expression; for example, the increase in fat content in Jian carp was associated with elevated expression of the fatty acid synthase (FAS) gene, whereas the decrease in fat content in female trout may reflect a

metabolic shift toward lean tissue growth (Zhao *et al.,* 2019; Öz *et al.,* 2025). Although L-glutamic acid did not alter muscle chemical composition under the present experimental conditions, these findings indicate that it has the potential to modulate protein and lipid metabolism under different physiological or nutritional scenarios.

Most essential and non-essential amino acids in muscle, including histidine, isoleucine, leucine, phenylalanine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, serine, and tyrosine, did not differ significantly among treatments. This finding is consistent with Belghit *et al*. (2023), who reported that glutamic acid and glycine supplementation did not significantly affect whole-body amino acid composition in rainbow trout, possibly reflecting sufficient amino acid levels in the basal diet or homeostatic mechanisms that maintain amino acid balance in fish. In the present study, however, cysteine content was significantly lower in the control group than in the other treatments. This finding is consistent with Zhao *et al.* (2019), who reported that glutamic acid supplementation increased cysteine levels and improved protein retention in Jian carp, likely because L-glutamic acid serves as a precursor for cysteine synthesis via metabolic pathways such as transamination. Lysine, methionine, and proline levels decreased in the 1% glutamic acid treatment. This reduction may reflect increased catabolism of these amino acids for energy production or to support glutamine synthesis, as Öz and İnanan (2025) reported that glutamine can become conditionally essential under stress conditions, thereby affecting amino acid metabolism. By comparison, Zhao *et al*. (2025) reported that glutamic acid supplementation increased lysine and methionine levels in largemouth bass; the discrepancy with the present study may reflect species differences or the higher supplementation level used in that study (up to 62.15%), which improved the essential amino acid profile. Furthermore, Ma *et al*. (2020) reported that glutamine supplementation increased proline levels in grass carp muscle. This discrepancy may reflect the role of glutamine in collagen synthesis and in enhancing the profile of flavor-related amino acids, whereas the decrease in proline observed in the present study may indicate a metabolic adjustment to maintain overall balance.

Regarding amino acid groups and ratios in muscle, total amino acids, essential amino acids, non-essential amino acids, branched-chain, aromatic, flavor-related, and acidic amino acids did not differ significantly among treatments ($p \geq 0.05$). This stability is consistent with Belghit *et al.* (2023), who reported that glutamic acid supplementation did not alter the overall body amino acid profile, likely because isonitrogenous diets maintain amino acid balance. Total sulfur-containing amino acids, however, were lower in the 1% L-glutamic acid treatment. This reduction may indicate metabolic competition with glutamic acid for sulfur-synthesis pathways, consistent with Zhao *et al.* (2019), who reported that glutamic acid supplementation increased overall essential amino acid levels but had

variable effects on specific groups such as sulfur-containing amino acids. The highest total basic amino acid content was recorded in the 0.5% treatment, consistent with Cai *et al*. (2025), who reported that glutamic acid supplementation increased basic amino acids such as arginine, possibly by enhancing protein synthesis via the IGF-1–AKT–mTOR pathway. Furthermore, the ratios of essential amino acids to total amino acids, essential to non-essential amino acids, and leucine to isoleucine were lowest in the 1% treatment. In contrast, the ratios of non-essential amino acids to total amino acids and flavor-related amino acids to total amino acids were lowest in the 0.5% treatment. These shifts may reflect the influence of glutamic acid on branched-chain amino acid metabolism, as Ma *et al.* (2020) reported that glutamine improved flavor-related ratios but may alter amino acid balance at moderate inclusion levels. In contrast, Zhao *et al*. (2025) reported that glutamic acid supplementation increased the ratio of essential to total amino acids; this discrepancy may relate to the optimal supplementation level identified in that study (51.12 g/kg), which improved growth but adversely affected some ratios, possibly reflecting species differences. Additionally, Öz and İnanan (2025) reported that glutamine affects amino acid metabolism under stress conditions, which may explain the stability of the major amino acid groups observed in the present study, where supplementation maintained overall balance while influencing specific amino acid ratios. In conclusion, moderate dietary L-glutamic acid levels can modulate the muscle amino acid profile; careful adjustment of the inclusion level is therefore needed to prevent adverse changes in amino acid ratios, with practical implications for aquaculture feed formulation.

The evaluation of physical quality parameters in rainbow trout muscle indicated that L-glutamic acid supplementation influenced color, texture, water-holding capacity, and cooking loss. These findings are in partial agreement with those of several studies on different fish species, although some variations were observed. The highest lightness (L*) was recorded in the 0.5% glutamic acid treatment, whereas the remaining color indices *a**, *b**, hue, and chroma did not differ significantly among treatments. This stability is consistent with Zhao *et al.* (2019), who reported that glutamic acid supplementation increased muscle pH but had no direct effect on color in Jian carp, possibly reflecting its role in reducing protein degradation and preserving myofibrillar structure, which in turn affects light scattering. The greatest total color change, however, occurred in the 2% treatment, which may be attributable to metabolic changes such as increased muscle fat at higher supplementation levels, consistent with Zhao *et al*. (2025), who reported that glutamic acid supplementation altered fat composition and affected physical traits such as pH and drip loss in largemouth bass. In the texture analysis, the highest hardness, gumminess, and chewiness occurred in the 2% treatment, consistent with Cai *et al*. (2025), who reported that glutamic acid supplementation increased hardness and springiness in gibel carp by improving

myofiber density and collagen content through activation of the IGF-1–AKT–mTOR pathway and regulation of myogenic factors such as MyoD and Myf5. The highest springiness occurred in the 1% treatment, consistent with Öz and İnanan (2025), who reported that glutamine supplementation improved fillet texture in female trout by reducing oxidative stress and enhancing antioxidant status, thereby affecting tissue structure. Regarding water-holding capacity, the highest value occurred in the 1% treatment, consistent with Zhao *et al.* (2025), who attributed reduced drip loss and increased pH following glutamic acid supplementation to lower lactate content and lactate dehydrogenase activity, resulting in improved water retention within the protein structure. The lowest cooking loss also occurred in this treatment, consistent with Ma *et al.* (2020), who reported that glutamine supplementation improved water-holding capacity. Overall, these results indicate that moderate to high dietary L-glutamic acid levels (1–2%) enhance muscle physical quality by improving myofibrillar structure, collagen content, and metabolic balance, whereas very high levels may increase color changes, underscoring the need for precise dietary adjustment. Among the tested levels, fish fed 1% L-glutamic acid exhibited the highest water-holding capacity and the lowest cooking loss, indicating superior muscle quality relative to the other treatment levels.

Conclusion

The findings of the present study indicate that dietary L-glutamic acid supplementation elicited responses that varied with inclusion level. Supplementation at 0.5% resulted in the highest activities of superoxide dismutase, catalase, and glutathione peroxidase, as well as the highest reduced glutathione levels, and was associated with differences in the concentrations of certain essential amino acids compared with the other treatments. In contrast, the 1% and 2% inclusion levels significantly improved the feed conversion ratio. Dietary L-glutamic acid supplementation also improved physical flesh quality, without significant changes in proximate composition or overall amino acid composition. From an applied perspective, the 1% inclusion level may represent a practical option for improving feed utilization while maintaining flesh quality in rainbow trout production. However, further validation under commercial farming conditions is required before firm recommendations can be made regarding optimal inclusion levels and economic benefits. Several limitations should be acknowledged, including the relatively short experimental duration, the absence of molecular and gene-expression analyses, and the evaluation of only one species and life stage. Future studies should investigate the long-term effects of L-glutamic acid supplementation, examine potential regulatory mechanisms related to antioxidant defense and protein metabolism, and assess its interactions with different dietary formulations and environmental stressors.

Conflicts of interest

The authors assert that no conflicting interests or personal relationships could

have affected the findings presented in this manuscript.

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